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## CLAIMS

*What is claimed is:*

1. A method of identifying a nucleic acid molecule suitable for use in a probe for detecting the presence of one or more of a family of nucleic acid molecules,
  - 5 comprising the steps of:
    - (a) providing the family of first nucleic acid molecules wherein each member of the family is related to all other members of the family by consensus sequence;
    - (b) providing a second nucleic acid molecule having a sequence  
10 complementary to the consensus sequence;
    - (c) determining the ability of the second nucleic acid molecule to form a duplex with each member of the family in the presence of a first ligand known to affect duplex formation of nucleic acid molecules;
    - (d) repeating step (c) for a plurality of concentrations of the ligand,  
15 wherein the nucleic acid molecule suitable for use in a probe is identified in step (c) at a ligand concentration at which the ability of the second nucleic acid molecule to form a duplex with each member of the family is substantially the same as its ability to form a duplex with each other member of the family.
2. The method of claim 1, further comprising the step of (e) repeating steps (c) and  
20 (d) for a second ligand.
3. The method of claim 2, further comprising the step of (f) repeating steps (c) and (d) in the presence of both first and second ligands.
4. The method of claim 3 wherein step (e) is repeated for each of a plurality of the second ligands and step (f) is repeated for each of the second ligands or combinations  
25 thereof.
5. The method of claim 1, further comprising the step of (g) determining the percent homology of the second nucleic acid sequence against a plurality of nucleic acid sequences in a database prior to step (c), wherein the second nucleic acid

sequence is less than a predetermined homology to other non-target partially complementary nucleic acid sequences.

6. The method of claim 1, wherein the first and second ligands are selected from the group consisting of actinomycin D, distamycin A, diminazane aceturate,  
5 bisbenzimidazole, and ethidium bromide.

7. The method of claim 1, wherein said first nucleic acid molecules of the family are at least a% homologous with each other, a being a number greater than 0 and less than 100, comprising the further steps of:

(i) providing a third nucleic acid molecule which is no more than b%  
10 homologous with each of the first nucleic acid molecules of the family, where b is a number greater than 0 and less than a;

(ii) repeating steps (c) and (d) in the presence of the third nucleic acid molecule so as to:

(a) determine the ability of the second nucleic acid molecule and  
15 the third nucleic acid molecule to form a duplex with each other, and

(b) determine a ligand concentration at which the ability of the second nucleic acid molecule to form a duplex with the third nucleic acid molecule is substantially different from the ability of the second nucleic acid molecule to form a duplex with each other member of the family,

20 wherein the second nucleic acid molecule is suitable for use as a probe when the ligand concentration at which the ability of the second nucleic acid molecule to form a duplex with the third nucleic acid molecule is substantially different from the ability of the second nucleic acid molecule to form a duplex with each other member of the family and the ligand concentration at which the ability of the second nucleic acid molecule to form a duplex with each member of the family is substantially the  
25 same as its ability to form a duplex with each other member of the family are substantially equal to each other.

8. The method of claim 7 wherein the second nucleic acid molecule is suitable for use as a probe when the ligand concentration at which the ability of the second nucleic acid molecule to form a duplex with the third nucleic acid molecule is substantially  
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less than the ability of the second nucleic acid molecule to form a duplex with each other member of the family and the ligand concentration at which the ability of the second nucleic acid molecule to form a duplex with each member of the family is substantially the same as its ability to form a duplex with each other member of the family are substantially equal to each other.

9. The method of claim 7, further comprising the step of (e) repeating steps (c), (d), (i) and (ii) for a second ligand.

10. The method of claim 9, further comprising the steps of (c), (d), (i) and (ii) in the presence of both the first and second ligands.

11. The method of claim 1 wherein each of the first nucleic acid molecules is selected from a group consisting of a genetic sequence of a first virus and variants thereof known to exist in nature.

12. The method of claim 7 wherein each of the nucleic acid sequences of the first nucleic acid molecules is selected from a genetic sequence of a first virus and variants thereof, and the nucleic acid sequence of the third nucleic acid molecule is selected from a group of genetic sequences known to exist in nature exclusive of the first virus and the variants.

13. A probe having a nucleotide sequence of the second nucleic acid identified for use in a probe according to any preceding claim.

14. A method of detecting the presence of a nucleic acid molecule suspected of being in a sample containing genetic material, the method comprising:

(A) providing the sample which contains or possibly contains a nucleic acid molecule which is a member of a family of first nucleic acid molecules that is related to all other members of the family by a consensus sequence;

(B) exposing the sample to a probe comprising a nucleotide sequence having a sequence complementary to the consensus sequence and identified according to the method of claim 1 as suitable for use in a probe, under conditions suitable for hybridization, in the presence of the ligand of claim 1

present at the concentration at which the ability of the second nucleic acid molecule to form a duplex with each member of the family is substantially the same as its ability to form a duplex with each other member of the family; and

(C) ascertaining whether any duplexed nucleic acid molecules comprising the probe formed in step (C), wherein the formation of the duplexed nucleic acid molecules indicates the presence of the nucleic acid molecule suspected of being in the sample.

15. The method of claim 14, comprising the further step of exposing the same of step (B) to conditions suitable for amplifying the duplex formed in step (B).

10 16. A method of promoting the hybridization of a nucleic acid capture moiety to a target single-stranded nucleic acid sequence and all its family members without hybridizing to a plurality of other non-target partially complementary nucleic acid sequences present in a sample, the steps comprising:

(a) providing:

15 (i) a nucleic acid capture moiety comprising the nucleic acid molecule identified in claim 5;

(ii) the sample containing the target duplex nucleic acid sequence or any of its family members, wherein the sample has been treated such that all duplex nucleic acid sequences present in the sample including the target duplex nucleic acid sequence and all its family members suspected of being present in the sample will denature and form single-stranded nucleic acid sequences; and

20 (iii) a nucleic acid sequence binding ligand;

(b) forming a reaction mixture comprising the nucleic acid capture moiety, the sample and binding ligand of steps (a)(i), (a)(ii) and (a)(iii), respectively under conditions such that the nucleic acid sequence binding ligand promotes hybridization of the target single-stranded nucleic acid sequence and all its family members to the nucleic acid capture moiety comprising the probe nucleic acid sequence identified in step (a) and not to other non-target partially complementary nucleic acid sequences;

25 (c) allowing the target single-stranded nucleic acid sequence and all its family members suspected of being present in a sample to hybridize to the nucleic  
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acid capture moiety comprising the probe nucleic acid sequence identified in step (a) such that the nucleic acid sequence binding ligand promotes hybridization of the target single-stranded nucleic acid sequence and all its family members to the nucleic acid capture moiety comprising the probe nucleic acid sequence identified in step (a) without promoting the hybridization of other non-target partially complementary nucleic acid sequences; and

(d) detecting the presence or absence of the target single-stranded nucleic acid sequence and all its family members hybridized to the nucleic acid capture moiety.

10 17. A method of promoting the hybridization of a nucleic acid capture moiety to a target single-stranded nucleic acid sequence and all its family members without hybridizing to a plurality of other non-target partially complementary nucleic acid sequences contained in a sample, the steps comprising:

(a) identifying at least one nucleic acid sequence probe substantially complementary to the target duplex nucleic acid sequence and all its family members suspected of being present in a sample and coupling at least one probe nucleic acid sequence with a label;

(b) identifying at least two substantially complementary primer nucleic acid sequences immediately 5' and 3' of the region of the target nucleic acid sequence and all its family members to be detected;

(c) treating the sample suspected of containing the target duplex nucleic acid sequences and all its family members with the two substantially complementary primer nucleic acid sequences in step (b), an agent for polymerization, and four nucleoside triphosphates under conditions which allow amplification of the nucleic acid sequence to be detected thereof without simultaneous amplification of non-target partially complementary nucleic acid sequences from other viral nucleic acid sequences or human genomic nucleic acid sequences;

(d) treating the sample such that all duplex nucleic acid sequences present in the sample including the amplified target duplex nucleic acid sequence and all its

family members suspected of being present in the sample denature and form single-stranded nucleic acid sequences;

(e) providing:

(i) a nucleic acid capture moiety comprising the labeled probe nucleic acid sequence identified in claim 5;

(ii) the sample suspected of containing the amplified single-stranded target nucleic acid sequence and all its family members; and

(iii) a nucleic acid sequence binding ligand;

(f) forming a reaction mixture comprising:

(i) the nucleic acid capture moiety comprising the labeled probe nucleic acid sequence identified in claim 5;

(ii) the sample suspected of containing the amplified single-stranded target nucleic acid sequence and all its family members; and

(iii) a nucleic acid sequence binding ligand;

under conditions such that the nucleic acid sequence binding ligand promotes hybridization of the amplified target single-stranded nucleic acid sequence and all its family members to the nucleic acid capture moiety comprising the probe nucleic acid sequence identified in step (a) and not to other non-target partially complementary nucleic acid sequences;

(g) allowing the amplified target single-stranded nucleic acid sequence and all its family members suspected of being present in a sample to hybridize to nucleic acid capture moiety comprising the probe nucleic acid sequence identified in step (a) such that the nucleic acid sequence binding ligand promotes hybridization of the target single-stranded nucleic acid sequence and all its family members to the nucleic acid capture moiety comprising the probe nucleic acid sequence identified in step (a) without promoting the hybridization of other non-target partially complementary nucleic acid sequences; and

(h) detecting the presence or absence of the target single-stranded nucleic acid sequence and all its family members hybridized to the nucleic acid capture moiety.

18. A hybridization mixture for promoting the hybridization of a nucleic acid capture moiety to a target single-stranded nucleic acid sequence and all its family members without hybridizing to a plurality of other non-target partially complementary nucleic acid sequences suspected of being present in a sample comprising:

(a) a nucleic acid capture moiety comprising a labeled probe nucleic acid sequence substantially complementary to the target duplex nucleic acid sequence and all its family members suspected of being present in a sample;

(b) at least one nucleic acid sequence binding ligand, wherein the ligand can promote hybridization of the target single-stranded nucleic acid sequence and all its family members to the nucleic acid capture moiety and not to other non-target partially complementary nucleic acid sequences.

19. The method of claim 17 wherein the nucleic acid sequence binding ligand is selected from the group consisting of: a compound which binds to a duplex nucleic acid in a sequence-specific way; a compound which binds to a duplex nucleic acid in a non-specific way; a protein; an enzyme; an enzyme which alters the structure of a duplex nucleic acid to which it binds; an enzyme which alters the structure of a duplex nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves one or both strands of a duplex nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the duplex to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; a nucleic acid ligase such as DNA ligase, an enzyme which promotes or catalyzes the synthesis of nucleic acid; a nucleic acid polymerase; a nucleic acid polymerase which requires a double stranded primer; a DNA polymerase; DNA polymerase I; Taq polymerase; an RNA polymerase; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding agent; a mutagen; a compound which enhances the expression of a gene under the control of the duplex bound by a ligand; a compound which interrelates into a duplex nucleic acid molecule; a compound which,



when contacted with a reaction mixture comprising a first single stranded nucleic acid molecule and a second single stranded nucleic acid molecule will increase the free energy of duplex formation at least  $n$ -fold, wherein  $n$  is 2, 5, 10, 50, 100, 500,  $10^3$ ,  $10^4$ ,  $10^5$ ,  $10^6$ , a compound which, when contacted with a reaction mixture will decrease the free energy of duplex formation by at least  $n$ -fold, wherein  $n$  is 2, 5, 10, 50, 100, 500,  $10^3$ ,  $10^4$ ,  $10^5$ ,  $10^6$ .

20. The hybridization mixture of claim 18, wherein the nucleic acid sequence binding ligand is selected from the group consisting of: a compound which binds to a duplex nucleic acid in a sequence-specific way; a compound which binds to a duplex nucleic acid in a non-specific way; a protein; an enzyme; an enzyme which alters the structure of a duplex nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves one or both strands of a duplex nucleic acid to which it binds, a restriction enzyme; a restriction endonuclease; an enzyme which methylates the duplex to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; a nucleic acid ligase such as DNA ligase; an enzyme which promotes or catalyzes the synthesis of nucleic acid; a nucleic acid polymerase; a nucleic acid polymerase which requires a double stranded primer; a DNA polymerase; DNA polymerase I; Taq polymerase, an RNA polymerase, an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding agent; a mutagen; a compound which enhances the expression of a gene under the control of the duplex bound by a ligand; a compound which intercalates into a duplex nucleic acid molecule; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid molecule and a second single stranded nucleic acid molecule will increase the free energy of duplex formation at least  $n$ -fold, wherein  $n$  is 2, 5, 10, 50, 100, 500,  $10^3$ ,  $10^4$ ,  $10^5$ ,  $10^6$ , a compound which, when contacted with a reaction mixture will decrease the free energy of duplex formation by at least  $n$ -fold, wherein  $n$  is 2, 5, 10, 50, 100, 500,  $10^3$ ,  $10^4$ ,  $10^5$ ,  $10^6$ .

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21. The method of claim 19, wherein the nucleic acid sequence binding ligand further comprises a single-stranded nucleic acid binding ligand.
22. The method of claim 19, wherein the nucleic acid sequence binding ligand further comprises a duplex nucleic acid sequence binding ligand.
- 5 23. The method of claim 19, wherein the nucleic acid sequence binding ligand further comprises a nonspecific nucleic acid binding ligand.
24. The method of claim 19, wherein the duplex nucleic acid sequence binding ligand is selected from the group consisting of actinomycin D, distamycin A, diminazene aceturate, bisbenzamide, and ethidium bromide.
- 10 25. The hybridization mixture of claim 20, wherein the nucleic acid sequence binding ligand further comprises a single-stranded nucleic acid binding ligand.
26. The hybridization mixture of claim 20, wherein the nucleic acid sequence binding ligand further comprises a duplex nucleic acid sequence binding ligand.
28. The hybridization mixture of claim 20, wherein the nucleic acid sequence
- 15 binding ligand further comprises a nonspecific nucleic acid binding ligand.
29. The hybridization mixture of claim 20, wherein the duplex nucleic acid sequence binding ligand is selected from the group consisting of actinomycin D, distamycin A, diminazene aceturate, bisbenzamide, and ethidium bromide.
30. The method of claim 17, wherein the nucleic acid capture moiety comprises a
- 20 structure A-B-C-D wherein:
- A is a single-stranded nucleic acid sequence having a composition substantially complementary to a region of the target nucleic acid sequence and all its family members to be detected;
- B and D are nucleic acid sequences which are capable of hybridizing to
- 25 each other to form an intramolecular duplex; and
- C is a linker which covalently links B and D.
31. The hybridization mixture of claim 18, wherein the nucleic acid capture moiety comprises a structure A-B-C-D wherein:

A is a single-stranded nucleic acid sequence having a composition substantially complementary to a region of the target nucleic acid sequence and all its family members to be detected;

5 B and D are nucleic acid sequences which are capable of hybridizing to each other to form an intramolecular duplex; and

C is a linker which covalently links B and D.

32. The method of claim 17 or 18, wherein the target nucleic acid sequence is detected by means of a labeled probe nucleic acid sequence, wherein the label is selected from the group consisting of antibody, antigen, radioisotope, fluorescent,  
10 enzyme, lectin or biotin.

33. The hybridization mixture of claim 19, wherein the label of the labeled probe nucleic acid sequence is selected from the group consisting of antibody, antigen, radioisotope, fluorescent, enzyme, lectin or biotin.

34. A kit for detecting the presence of a target nucleic acid sequence and all its  
15 family members suspected of being present in a sample, the kit comprising:

(a) a nucleic acid capture moiety comprising a labeled probe nucleic acid sequence substantially complementary to the target duplex nucleic acid sequence and all its family members suspected of being present in a sample; and

(b) at least one nucleic acid sequence binding ligand, wherein the ligand can  
20 promote hybridization of the target single-stranded nucleic acid sequence and all its family members to the nucleic acid capture moiety and not to other non-target partially complementary nucleic acid sequences.

34. The kit of claim 33, further comprising at least one component selected from the group consisting of a positive control containing one or more nucleic acid sequences  
25 with a sequence of the target nucleic acid sequence and all its family members, a negative control which does not contain any nucleic acid sequences containing the sequence containing the suspected target nucleic acid sequence and all its family members.

35. The kit of claim 33, further comprising two substantially complementary primer nucleic acid sequences immediately 5' and 3' of the target nucleic acid sequence and all its family members, an agent for polymerization, and each of four nucleoside triphosphates.
- 5 36. The kit of claim 33, wherein the agent for polymerization is an enzyme selected from the group consisting of E. coli DNA polymerase 1, Klenow fragment of E. coli DNA polymerase, reverse transcriptase, or a thermostable DNA polymerase.
37. The kit of claim 33, wherein the target nucleic acid sequence and all its family members is a DNA sequence, the primer nucleic acid sequences are
- 10 oligodeoxyribonucleotides, and the nucleoside triphosphates are dATP, dCTP, dGTP, and dTTP.
38. The kit of claim 34, wherein the positive control is a region of viral nucleic acid sequence to be detected. ~~X~~
39. The kit of claim 38, wherein the viral nucleic acid sequence to be detected is a
- 15 region of the AIDS virus and all its family members.
40. The method of claim 17 wherein the target nucleic acid sequence and all its family members to be detected is a region of a viral nucleic acid sequence and all its family members.
41. The method of claim 40, wherein the region of viral nucleic acid to be detected
- 20 is a region of the AIDS virus and all its family members.
42. The method of claim 17, wherein the target nucleic acid sequence and all its family members to be detected is a region of an oncogene.
43. The method of claim 47, wherein the oncogene is selected from the group consisting of p53, ras, BRCA1, or BRCA2 and each of their family members.
- 25 44. The kit of claim 33, wherein the labeled probe nucleic acid sequence is selected from the group consisting of p53, ras, BRCA1, or BRCA2 and each of their family members.

45. The kit of claim 34, wherein the positive control is a region of the oncogene and all its family members to be detected.

46. The kit of claim 45, wherein the oncogene and all its family members to be detected is selected from the group consisting of p53, ras, BRCA1, BRCA2, or APC.

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